



Tissue Microrna As a Potential Biomarker in Oral Squamous Cell Carcinoma - A Systematic Review

Amritha Sripoo.R¹, Uma Maheswari T.N^{2*}

¹Postgraduate, Oral Medicine And Radiology, Saveetha Dental College And Hospitals, Saveetha Institute Of Medical And Technical Sciences, Saveetha University, Chennai-6000077

²Professor And Head Of Admin, Department Of Oral Medicine And Radiology, Saveetha Dental College And Hospitals, Saveetha Institute Of Medical And Technical Sciences, Saveetha University, Chennai:6000077

***Corresponding author:** Uma Maheswari T.N, Professor And Head Of Admin, Department Of Oral Medicine And Radiology, Saveetha Dental College And Hospitals, Saveetha Institute Of Medical And Technical Sciences, Saveetha University, Chennai:6000077,
Email: Umamaheswaritn@Saveetha.Com

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ABSTRACT

Background: Oral squamous cell carcinoma is the most common form of carcinoma of the oral cavity and ranks as the 12th most common cancer in the world. Cancer development and progression requires inactivation of tumour suppressor gene and activation of proto-oncogenes. Expression of these genes is in part dependent on RNA –based mechanisms. MicroRNAs are essential regulators of diverse cellular processes including proliferation, differentiation ,apoptosis ,survival, motility , invasion and morphogenesis

Aim: To evaluate the differential expression of tissue miRNAs in OSCC tissue samples and their use as biological markers to predict the prognosis of OSCC patients.

Methods: A search was done using MeSH terms and keyword search in the electronic databases namely PubMed, Google Scholar, Cochrane, Science Direct ,Lilacs and additional searches were carried out through cross checking the bibliographies of selected articles. Then based on the inclusion and exclusion criteria and availability of the full texts, a total of 2 articles were included in this systematic review

Result: The search yielded a total of articles out of which 56 articles were included based on the eligibility criteria. Quality assessment based on the Quality Assessment of Diagnostic Accuracy Studies 2 tool was used to obtain a risk of bias chart using Revman 5.4 software and it was proved that from the 2 included studies, one had low risk of bias and another had moderate risk of bias.

Conclusion: This systematic review aimed at improving the current understanding of next generation sequencing and applicability in OSCC. Further, this may be an alleyway for the identification of newer biomarkers using NGS-based technique.

Keywords: *miRNA, Oral Squamous Cell Carcinoma, Biomarker*

INTRODUCTION

MiRNA plays an important role in cellular growth, differentiation, apoptosis, and immune response, while some miRNAs aid in tumour suppression.(1)(2) During development of malignancy, some miRNAs are upregulated and some are downregulated, so any change in the expression of miRNAs can cause tumour suppression or act as carcinogens.(3)Victor Ambros et al., Rosalind Lee and Rhonda Feinbaum were the first to discover miRNA. Several hundred genes in our genome encode small functional RNA molecules collectively called miRNAs and are found in normal tissues, blood, and saliva.(4)

Oral Squamous Cell Carcinoma is the most common malignant epithelial neoplasm affecting the oral cavity.(5,6)(7)The Indian subcontinent accounts for one-third of the oral cancer burden in the world.Oral Squamous Cell Carcinoma(OSCC) is the most common malignant epithelial neoplasm affecting the oral cavity.(8)OSCC accounts for 90%- 96% incidence of whole head and neck cancers and the Survival rate is about 50% of affected cases. Prediction of survival in oral cancer depends on classical parameters such as tumour grade and depth of invasion.Although many biomarkers have been introduced as potential prognosticators of OSCC,there are no sensitive biomarkers for detection of OSCC.(9)(10).Any change in the expression of miRNAs can cause tumour suppression or act as carcinogens.Survival rate of oral squamous cell carcinoma (OSCC) is about 50% of affected cases.(10) Prediction of survival in oral cancer depends on classical parameters such as tumour grade and depth of invasion, although many biomarkers have been introduced as potential prognosticators of OSCC.site specific analysis.(5)(11)

In this systematic review, various studies done in tissue miRNA as a biomarker for oral squamous cell carcinoma were included in this study.This paves the way for future study.

MATERIALS AND METHODS

Study design & search methodology

For this study, we followed the guidelines given by the Preferred Reporting Items for Systematic

Reviews and Meta-Analyses Guidelines (PRISMA).

The studies included in this systematic review were identified by a comprehensive search from the following search engines using the keywords.PubMed Advanced search using MeSH terms ,Google scholar,Cochrane ,ScienceDirect,Latin American and Caribbean Health Sciences Literature (LILACS) upto Hand searching of relevant articles was done until September 2022 [Figure 01]

Search strategy

For the search strategy, MeSH terms and free text words were combined through Boolean operators: (((miRNAs OR microRNAs OR miRNA OR microRNA))) AND ((OSCC OR 'oral squamous cell carcinoma' OR 'oral cancer' OR 'Tongue Cancer' OR TSCC OR OTSCC OR Gingiva OR 'Head Neck'))

Eligibility criteria

Inclusion criteria

- 1.Studies written in the English language
- 2.Full text articles
- 3.Reporting about the evaluation of miRNA expression in a cohort of at least 30 patients.
- 4.Quantification of the expression made on tissue samples by quantitative polymerase chain reaction (qPCR), in situ hybridization (ISH), fluorescent in situ hybridization (FISH), or RNA sequencing

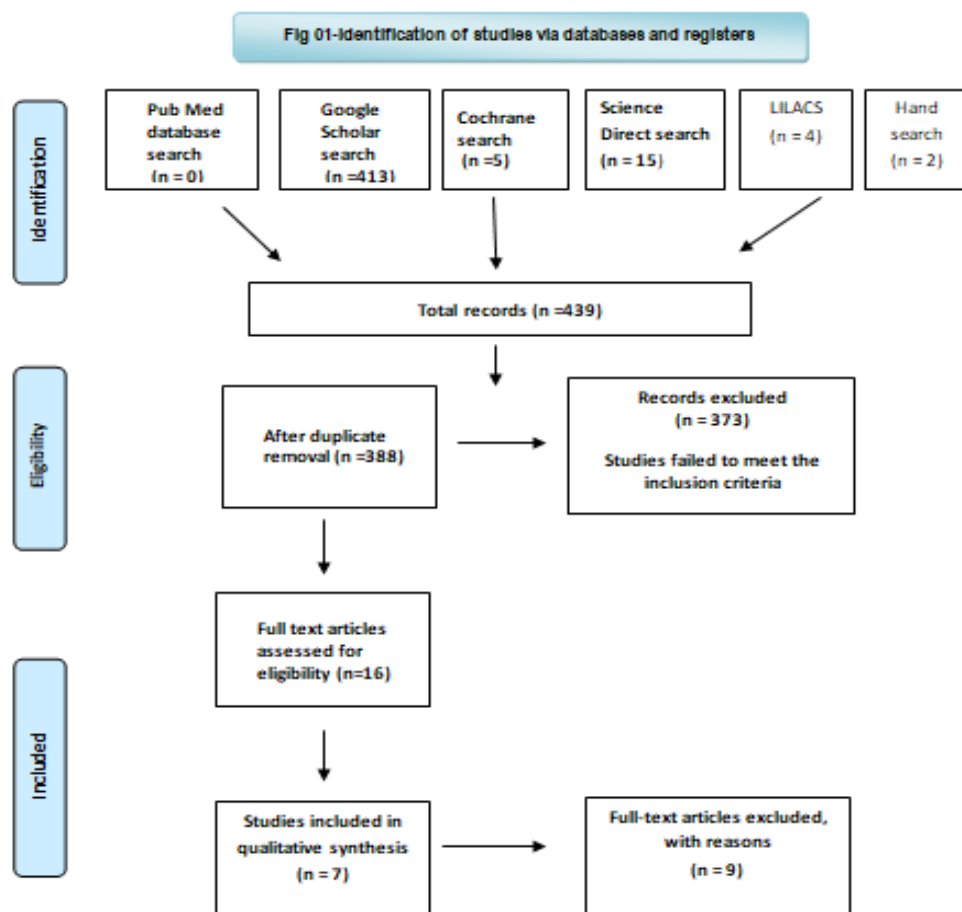
Exclusion criteria

- 1.Animal studies were excluded
- 2.Studies done on other carcinoma other than OSCC were excluded
- 3.Abstract only

Selection process

Two investigators independently evaluated articles retrieved from the databases. First round of evaluation was performed by reading only the title and abstracts of the studies. At the end of the first round all studies considered eligible were included for full-text evaluation. A direct search

of bibliographies of articles in full-text was carried out, to find out further articles to include. After full-text reading, only studies considered eligible by both authors were included.



Data extraction

Data extraction of the characteristics of included studies and the variables of outcome are given in (Table 01)

Author/Year	miRNAs investigated	Dysregulation	Samples	Follow-up	Site	Assay	Type of Samples	Cutoff	Survival analysis
Yu et al.,(2017)	miR-21	Up regulation	100	100 months	37 buccal mucosa;35 tongue;12 floor of the mouth;16 others	ISH	FFPE	Low (score = 1): 0%-33% positive cells; medium (score = 2): 34%-66% positive cells; high (score = 3): 64%-100% positive cells	Multivariate
Cheng et al.,(2016)	miR-455-5p	Up regulation	58	120 months	Not reported	qPCR	Fresh Frozen	Mean	Univariate

Yu et al.,(2016)	miR-204	Down regulation	80	60 months	46 tongue; 33 buccal mucosa; 1 other	qPCR	Fresh Frozen	Not reported	Univariate
Wu et al. (2016)	miR-101	Down regulation	40	60 months	Not reported	qPCR	Fresh Frozen	Not reported	Univariate
Baba et al. (2016)	miR-155-5p	Up regulation	73	60 months	34 tongue; 25 gingiva; 7 cheek; 4 floor of the mouth; 3 soft palate	qPCR	FFPE	Not reported	Multivariate
Jakob et al.(2019)	miR-99-3p.miR-100-5p	Up regulation	36	-	6 floor of mouth, 25 tongue, 5 palate	qPCR	Fresh Frozen	Mean	Multivariate
Yuanyuan et al , (2021)	miR-198	Downregulation	80	-	Not mentioned	qPCR	Fresh Frozen	Mean	Multivariate

Quality assessment of the studies

The quality of these 9 studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 tool. This tool includes 14 items which assess the risk of bias and sources of variation in diagnostic studies. It is recommended by the Cochrane Collaboration, Agency for

Health Care Research and Quality and the UK National Institute of Health and Clinical Excellence to assess the quality of diagnostic studies. QUADAS-2 is an improvised redesigned tool from the Cochrane Collaboration based on feedback from editors of the original QUADAS tool

Risk of bias

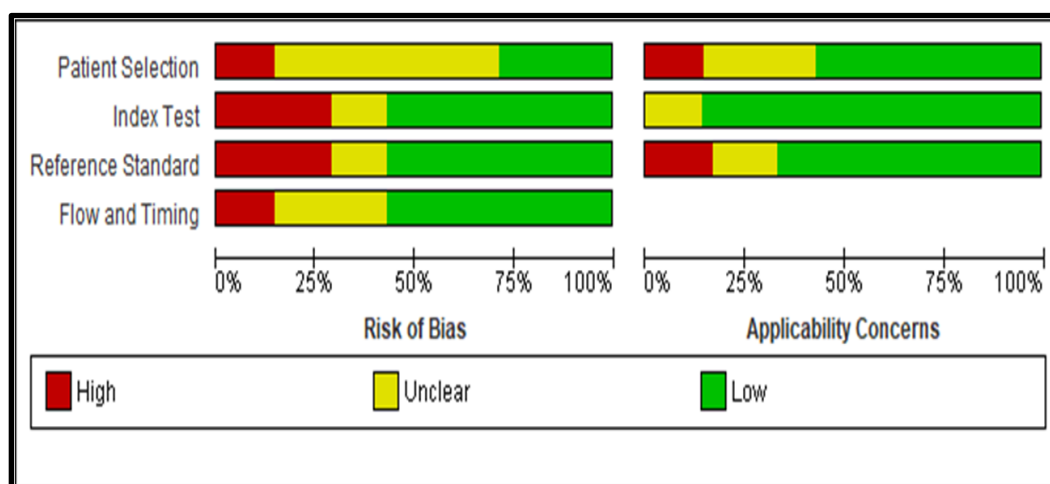


FIGURE 2 : Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

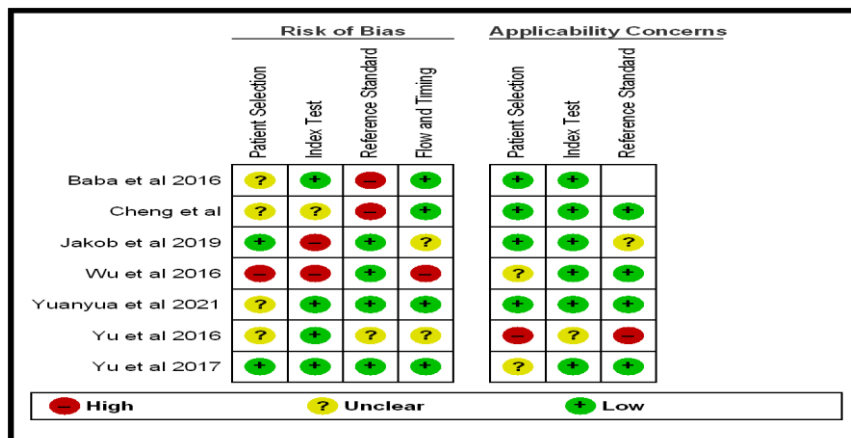


FIGURE 3 : Risk of bias graph: review authors' judgements about each risk of bias item across all included studies.

RESULT

Risk of bias assessment was performed using the RevMan5.4. Among the 7 studies, studies by Wu et al 2016, Yu et al 2016 showed moderate risk of bias, other 5 studies had low risk of bias. The pool of miRNAs derived from multivariate analysis exhibited a stronger correlation with poor prognosis compared to those from univariate analysis. Poor prognosis correlated with 4 miRNAs obtained (miRNA-21, miRNA-155-5p, miRNA-99-3P, 100-5P, miRNA-198). The results revealed that the expression levels of specific miRNAs can robustly predict prognosis of OSCC patients.

DISCUSSION

In the present study, we systematically analysed 7 suitable articles and reported. Despite improvements in diagnostic and therapeutic tools, the survival rate of patients with OSCC is still low. Thus, the possibility of offering personalised therapeutic schedules would benefit OSCC patients enormously. Biomarker is a term that defines different types of objective indicators of health or disease. (12)(13) Throughout history, and according to human technological advancements, these indicators have turned increasingly more precise and reliable. (14) Biomarkers predicting OSCC prognosis with some accuracy would be very useful for clinicians as this would allow choosing the magnitude and type of therapeutic approach (surgery, chemotherapy, radiotherapy, and a

combination of these) on the basis of the molecular profile of OSCC. (15)(16)(17) In the past few years, expression of miRNAs has been evaluated in several studies with a view of selecting potential diagnostic and/or prognostic biomarkers in solid tumours. (18) This study was to systematically review the literature about the use of miRNAs as prognostic clinical biomarkers in patients with OSCC. Among the included articles multivariate analysis is carried out only in 4 articles. Studies done on the expression of miR-21, miR-455-5p, miR-155-5p, miR-99-3p, miR-100-5p in oral squamous cell carcinoma showed upregulation, whereas studies done on the expression of tissue miR-204, miR-101, miR-198 showed downregulation in oral squamous cell carcinoma.

In a previous study by G. Troiano et al., a total of 15 studies featuring 1,200 OSCC samples, predominantly from Asia, met the inclusion criteria and were included in the meta-analysis. Poor prognosis correlated with upregulation of 9 miRNAs (miR-21, miR-455-5p, miR-155-5p, miR-372, miR-373, miR-29b, miR-1246, miR-196a, and miR-181) and downregulation of 7 miRNAs (miR-204, miR-101, miR-32, miR-20a, miR-16, miR-17, and miR-125b). The pooled hazard ratio values (95% confidence interval) related to different miRNA expression for overall survival and disease-free survival were 2.65 (2.07–3.39) and 1.95 (1.28–2.98), respectively. (19)

Although the results of this systematic review is supported by strong evidence, some limitations deserve attention. It does not allow for comparison among components of the panel. A limitation is that we included studies that used different detection methods (qPCR and ISH) and types of tissues (FFPE and fresh frozen). Furthermore, OSCC in Asia shows distinctive patterns and appears to have specific carcinogenic mechanisms. The results revealed that the expression levels of specific miRNAs can robustly predict prognosis of OSCC patients. Our team has extensive knowledge and research experience that has translate into high quality publications. (20)(21,22)(23)(24)(11)

CONCLUSION

This systematic review identifies several miRNAs that correlate with poor prognosis in patients with OSCC and could potentially be used in clinical practice when adequate statistical power of the evidence will be achieved. Such molecules should be evaluated in combination with other clinical and molecular biomarkers to choose the best treatment option in patients with OSCC. This study encourages conducting further studies on human samples with the aim of increasing the power of evidence and to confirm our preliminary results.

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